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Transcriptional alterations under continuous or pulsatile dopaminergic treatment in dyskinetic rats

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Abstract Continuous dopaminergic treatment is considered to prevent or delay the occurrence of dyskinesia in patients with Parkinson's disease (PD). Rotigotine is a non-ergolinic $D_3 > D_2 > D_1$ dopamine-receptor agonist for the treatment of PD using a transdermal delivery system providing stable plasma levels. We aimed to investigate the differential influence on gene expression of pulsatile L-DOPA or rotigotine versus a continuous rotigotine treatment. The gene expression profile within the nigrostriatal system of unilateral 6-hydroxydopamine-lesioned rats was assessed in order to differentiate potential changes in gene expression following the various treatment using Affymetrix microarrays and quantitative RT-PCR. The expression of 15 genes in the substantia nigra and of 11 genes in the striatum was altered under pulsatile treatments inducing dyskinetic motor response, but was unchanged under continuous rotigotine treatment that did not cause dyskinetic motor response. The route of administration of a dopaminergic drug is important for the induction or prevention of motor abnormalities and adaptive gene expressions. The decline of neurotrophin-3 expression under pulsatile administration was considered of particular importance.

Keywords Continuous · Dyskinesia · Gene chip · Gene expression · 6-Hydroxydopamine · L-DOPA · Microarray · Parkinson's disease · Pulsatile · Quantitative RT-PCR · Rotigotine

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Introduction

The mainstay of treatment in Parkinson's disease (PD) has been palliative treatment for dopamine deficiency with L-DOPA (L-3,4-dihydroxyphenylalanine, levodopa) or dopamine agonists. However, after few years of treatment, the occurrence of motor complications limits the usefulness of especially L-DOPA (Jankovic 2005; Jenner 2008). It has been proposed that pharmacokinetic factors such as pulsatile drug administration and pharmacodynamic alterations in the brain of PD patients are causative factors (Nutt 2007; Cenci 2007). Recent studies suggest that pulsatile delivery of L-DOPA results in changes in gene and protein expression and in subsequent alterations of the firing patterns of neurons (Chase 2004; Valastro et al. 2007, Sgambato-Faure et al. 2005).

Rotigotine, is a non-ergolinic $D_3 > D_2 > D_1$ dopamine-receptor agonist for once daily treatment of PD formulated

in a patch providing a sustained drug release over 24 h (Baldwin and Keating 2007; Giladi et al. 2007; Steiger 2008). In the MPTP-induced PD in mice, continuous rotigotine administration reduced dyskinesia in comparison to pulsatile treatment with rotigotine or L-DOPA (Stockwell et al. 2010). Stable plasma levels are thought to maintain continuous dopaminergic stimulation and to prevent induction of dyskinesia.

In the present study, the unilaterally 6-hydroxydopamine (6-OHDA) lesioned rat (Jenner 2008) was used as a model of PD to investigate the relationship between adaptive gene expression changes and the occurrence of motor complications under different treatment regimen. Rats sustaining such lesions exhibit robust contraversive turning following dopaminergic treatment due to hypersensitization of the dopamine receptors ipsilateral to the lesion (Jenner 2008) and abnormal involuntary movements (AIMs, Cenci 2002; Cenci et al. 1998). Recently, we reported that the continuous in contrast to the pulsatile administration of rotigotine did not induce sensitization nor AIMs, suggesting that continuous stimulation of dopaminergic receptors by rotigotine has no or a low propensity to induce dyskinesia in this experimental model (Schmidt et al. 2008). Here, transcript profiling via microarray was used to examine the expression changes of over 1,200 sequences in the substantia nigra (SN) and striatum under continuous or pulsatile treatment conditions. We focused on the genes of the dopaminergic, serotonergic and glutamatergic system that are hypothesized to be involved in the pathogenesis of L-DOPA-induced dyskinesia and aimed at testing the hypothesis that the pulsatile administration of rotigotine or L-DOPA would lead to a differential gene expression compared to a continuous administration of rotigotine.

Materials and methods

Animal experiments

Animal experiments were carried out at the Department of Neuropharmacology (University of Tübingen). All procedures were done in accordance with the ethical principles and to the requirements of the German laws on animal experimentation. The study protocol was approved by the local ethical committee and the local legal authority.

6-OHDA lesions and behavioral testing

The induction of unilateral lesions and of behavioral testing is described elsewhere (Schmidt et al. 2008). In short, male Sprague–Dawley rats (BW 220–250 g, Charles River, Germany) housed under standard conditions (RT 22.5°C, relative humidity 55%; 12/12-h light/dark cycle with access to water ad libitum but limited access to food (15 g per animal and day). Under anesthesia with pentobarbital-sodium (Narcoren®, Merial Hallbergmoos, Germany, 64 mg/kg, i.p.) and desipramine hydrochloride (Sigma Taufkirchen, Germany, 20 mg/kg, i.p.), 6-OHDA hydrobromide (12 µg (free base dissolved in 1 µL ascorbic acid 0.01%, Sigma Taufkirchen, Germany) was infused into the left medial forebrain bundle at a rate of 0.25 µL/min [coordinates in respect to bregma: $A = 4.0$ mm, $L = 1.6$ mm, and $H = 8.8$ mm (Paxinos and Watson 1986)]. Retrograde degeneration of the dopaminergic cells developed over 29 days. Only those rats demonstrating a spontaneous ipsiversive rotation in a novel environment were included in the test which has been demonstrated as a clear indicator for an effective lesioning (Metz et al. 2005). A challenge test with a standard dose of L-DOPA or apomorphine had to be omitted, as even a single administration of either compound may have caused priming for sensitization or AIMs (Morelli et al. 1991).

Animal treatment

The rats were randomly divided in four experimental groups and were treated for 10 days (day 30–39 after surgery) followed by a 5 day drug-free period and a re-testing for confirmation of established sensitization and dyskinesia: group 1 [(GI, control) saline; group 2 (GII) 10 mg/kg L-DOPA and 15 mg/kg benserazide; group 3 (GIII) continuous rotigotine (1 mg/kg slow release formulation prepared as oily suspension of 0.1% rotigotine (SCHWARZ PHARMA); group 4 (GIV) pulsatile rotigotine (1 mg/kg, dissolved as 1.0 mg/mL in 1:15 DMSO/saline (SCHWARZ PHARMA)]. L-DOPA and rotigotine doses were selected based on consistent effects as observed in previous studies (Alam et al. 2004) and providing plasma levels similar to those achieved in humans (Elshoff et al. 2006).

Sample and array processing

Brain tissue preparation

On day 18 after onset of treatment (day 47 after surgery) the animals were deeply anesthetized with halothane and intubated. After 20 min, the brains were frozen in situ by liquid nitrogen poured into a skin funnel. The frozen brains were chiseled out and the striatum and SN region from the damaged side (left side) were dissected (−20°C in a cryostate chamber) and stored at −80°C.

Total RNA extraction

Total RNA was isolated from the dissected tissue and separately for each animal using RNeasy Midi Kit (Qiagen,

Hilden, Germany), following the procedure described by the reagent's manufacturer. For Gene Chip expression probe array hybridization, total RNA was pre-treated with DNase I as previously described (Grünblatt et al. 2004a, b). Additional quality test of the RNA, before going to the GeneChip arrays, was performed using the GeneChip® Test3 Array (Affymetrix, Santa Clara, USA).

cRNA labeling and array hybridization

Rat Neurobiology GeneChip array (Affymetrix, Santa Clara, USA), consisting of approximately >1,200 genes each (of known genes and ESTs) was employed. The RNA samples from the different animals were pooled for each treatment group and brain region (per pool ten samples) resulting with eight groups (four treatments, two regions). Pooling was performed in triplicates per treatment group, and per brain region. Labeled cRNA was prepared from the different pools of total RNA using GeneChip® Two-Cycle Target Labeling and Control Reagents. This technique utilized biotin-labeled and fragmented complementary RNA that was hybridized to the chip. Preparation of the cRNA and defragmentation were conducted according to the manufacturer's protocol (Affymetrix, Santa Clara, USA). The hybridization and scanning of the probe arrays were performed as previously described (Grünblatt et al. 2004a, b; Jacob et al. 2007).

Data analysis and criteria for gene selection

Results are averaged from triplicate independent array hybridization experiments. The data analysis was performed using Vector Xpression™ 3.0 Microarray Data Analysis Software (Invitrogen GmbH, Karlsruhe, Germany). Data of each array was normalized by dividing the median of its gene expression value. Data were then filtered based on the following criteria: (1) Hybridization was robust and therefore the gene's expression was "present" in at least one of the sample groups according to Affymetrix criteria, (2) hybridization levels were rated as "changed" according to MAS5 criteria, (3) the fold change ratio between medication group and controls was ≥ 1.5 ($\log_2 > 0.58$; significant up-regulation) or ≤ 0.67 ($\log_2 < -0.58$, significant down-regulation). Wilcoxon test was conducted for significance of the triplicate independent hybridization experiments.

Quantitative RT-PCR

Total RNA (1 μg for striatum, 100 ng for SN) from each sample was reverse transcribed with random hexamer and oligo-dT primers using iScript™ cDNA Synthesis Kit (BioRad Laboratories, Hercules, CA, USA, Cat. No. 170-8890). The genes were normalized to the house-keeping genes: β-actin, ribosomal 18S, α-tubulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table 1). The house-keeping genes were tested for their stability using the GeNorm program (http://medgen.ugent.be/~jvdesomp/genorm/) (Vandesompele et al. 2002). After analysis for the most stable house-keeping genes, a normalization factor was calculated according to the program GeNorm. Absence of DNA contamination was verified by amplifying the house-keeping gene, ribosomal 18S, and run on gel to observe no product. Minus RT samples were tested simultaneously with experimental samples by quantitative RT-PCR (Q-RT-PCR) in order to see whether the reaction did not yield any amplification below 35 cycles using the PCR protocol. Eight genes (Table 1) were assessed for their gene expression profiles in the SN and striatum from the left side. Q-RT-PCR was performed on the iCycler iQ system (BioRad Co., Hercules, CA, USA) as described previously (Grünblatt et al. 2004a). QuantiTect SYBR Green PCR Kit was used for the analysis of the various genes (Qiagen Inc., Valencia, CA, USA). All PCR reactions were run in triplicate and for each sample individually. A melting point analysis was conducted to further confirm analysis of one product detection on the Q-RT-PCR analysis. Standard curves for each amplification product were generated from tenfold dilution of pooled cDNA amplicons to determine primer efficiency and quantization. Amplicons were harvested from the first RT-PCR test of each gene and their extraction from agarose gel electrophoresis using the MiniElute Gel extraction kit (Qiagen). Data was analyzed with Microsoft Excel 2000 to generate raw expression values.

Data analysis

Results reported here are averaged from triplicate independent Q-RT-PCR experiments for the individual rat brain region. The amplified transcripts were quantified using the comparative threshold cycle (C_T) method. For the accurate normalization of Q-RT-PCR data the geometric averaging of multiple internal control genes (the most stable house-keeping genes) was used according to the program GeNorm (Vandesompele et al. 2002). Kruskal–Wallis One Way Analysis of Variance (ANOVA) on Ranks and pair wise multiple comparisons versus control group (Dunn's Method) was conducted for significance of the triplicate independent experiments. Analysis was conducted on the PC program Sigma Plot 9.0 (Systat Software GmbH, Erkrath, Germany). Significant differences were defined as those with a P value <0.05.

Table 1 QuantiTect primer assays conditions for real-time quantitative RT-PCR

Gene	Accession no.	Gene symbol	QuantiTect primer assay	Detection method	Product size (bp)	Cycle no.	Reaction efficiency (%)	Melting point (°C)
Ribosomal 18s	M11188	Rnr1	Cat nr: QT 00199374	SYBR	103	40	92.3	88
Beta-actin	NM_031144	Actx	Cat nr: QT 00193473	SYBR	145	37	98.1	85.5
Alpha-tubulin	NM_022298	Tuba1	Cat nr: QT 00195846	SYBR	85	40	100	85.5
Glyceraldehyde-3-phosphate dehydrogenase	NM_017008	Gapd	Cat nr: QT 00199633	SYBR	83	35	100	84.5
Neurotrophin 3	NM_031073	Ntf3	Cat nr: QT 00184373	SYBR	106	45	99	79
Insulin-like growth factor 2	NM_031511	Igf2	Cat nr: QT 00195594	SYBR	96	40	100	86,5
Tumor necrosis factor superfamily, member 2	NM_012675	Tnf	Cat nr: QT 00178717	SYBR	75	45	95.2	80.5
Glutamate receptor, metabotropic 2	XM_343470	Grm2	Cat nr: QT 00373030	SYBR	73	45	83.3	78.5
Solute carrier family 1; glial high affinity glutamate transporter; member 2	NM_017215	Slc1a2	Cat nr: QT 00181090	SYBR	91	40	93.2	81
Solute carrier family 6 neurotransmitter transporter, serotonin, member 4	NM_013034	Slc6a4	Cat nr: QT 00176827	SYBR	83	40	100	78
5-hydroxtryptamine receptor 1A	NM_012585	Htr1a	Cat nr: QT 00412643	SYBR	117	45	96.6	84
5-hydroxtryptamine receptor 2A	NM_017254	Htr2a	Cat nr: QT 00382893	SYBR	125	40	98.4	82

Results

Using stringent criteria for the selection of meaningful candidate genes, 1,150 out of 1,322 transcripts (87%) assessed reached Affymetrix criteria "present" and "changed" in the SN, while 829 out of 1,322 transcripts (63%) assessed reached Affymetrix criteria "present" and "changed" in the striatum. At a probability level below 0.05 ($P < 0.05$), 8.1 and 5.1% of these mRNAs were expressed at altered levels in the SN and striatum, respectively, of unilateral 6-OHDA-lesioned rats that developed dyskinetic motor response. The expression of 68 mRNAs was up- and that of 69 mRNAs was down-regulated within the SN whereas the expression of 33 mRNAs was up- and 35 mRNAs was down-regulated within the striatum.

Differentially expressed genes associated with the dopaminergic, serotoninergic and glutamatergic neurotransmission are summarized in Tables 2 and 3. Dysregulation of these neurotransmitter systems is potentially involved in the pathophysiology of dyskinesia (Jenner 2008; Brotchie 2005).

As depicted in Table 2, only 20 genes were changed in a similar way in the SN of unilateral 6-OHDA-lesioned rats that developed dyskinetic motor response following pulsatile L-DOPA- and rotigotine treatment. The transcripts of orotidine-5′-phosphate decarboxylase, retinal guanylate cyclase 2F, member 6 of the tumor necrosis factor superfamily, BH3 interacting domain, rat tumor necrosis factor, alpha type 2 of glutathione-S-transferase, glial high affinity glutamate transporter, metabotropic 2 and 5 glutamate receptor, ionotropic kainate 2 glutamate receptor, serotonin 5B and 1A receptor were up-regulated whereas guanylyl cyclase, BCL2-like 11 and the serotonin transporter were down-regulated. Interestingly, all these changes were either not changed due to the lesion and treatment or were normalized in those rats which received the continuous treatment with rotigotine.

In the striatum (Table 3), only 19 genes were changed in a similar way. The transcripts of insulin-like growth factor binding protein 5 (Accession no. rc_AI029920), insulin-like growth factor 2, fibroblast growth factor 5, metabotropic 2 glutamate receptor, serotonin 2A and 5A receptor and the serotonin transporter were up-regulated whereas

Table 2 Genes in the substantia nigra of unilateral 6-hydroxydopamine-lesioned rats that developed dyskinetic motor response following L-DOPA and pulsatile rotigotine treatment and are similarly changed

Gene name and functional group	Accession no.	Log2(GII/GI)	Log2(GIII/GI)	Log2(GIV/GI)
Genes involved in enzyme metabolism				
Orotidine-5′-phosphate decarboxylase	AFFX-YEL0-21w/URA3	**0.518**	0.368	**0.518**
Guanylyl cyclase (GC-E)	L36029	**−1.584**	0.120	**−0.891**
Guanylate cyclase 2F, retinal	L36030	**0.961**	−0.071	**0.915**
Genes involved in apoptosis				
Tumor necrosis factor receptor superfamily, member 6	AFFX-MurFAS	**0.853**	0.569	**1.380**
BH3 interacting (with BCL2 family) domain, apoptosis agonist	D83697	**1.111**	0.435	**0.586**
BCL2-like 11 (apoptosis facilitator)	AF065432	**−1.143**	0.312	**−1.459**
Rat tumor necrosis factor receptor (TNF receptor) mRNA, complete cds	M63122	**0.932**	0.360	**0.969**
Genes involved in the metabolism of reactive oxygen species				
Glutathione-*S*-transferase, alpha type2	rc_AI235747	**0.627**	0.096	**1.020**
Genes involved in neurotransmission				
Dopamine receptor 1A	S46131mRNA	0.248	**1.038**	0.537
Solute carrier family 1 (glial high affinity glutamate transporter), member 2	rc_AI044517	**1.742**	0.510	**1.801**
Glutamate receptor, metabotropic 5	D10891	**1.485**	0.516	**0.871**
Glutamate receptor, metabotropic 4	M90518	−0.102	**0.910**	−0.124
Glutamate receptor, metabotropic 2	M92075	**2.843**	0.110	**1.388**
Glutamate receptor, ionotropic, kainate 2	Z11548	**1.786**	0.126	**1.393**
Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 1	S39221	0.394	**0.781**	0.341
Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 1	L08228exon#22	0.388	**0.584**	0.172
Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 2A	AF001423	0.332	**0.686**	0.535
Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	X63995	**−0.909**	0.168	**−1.703**
5-hydroxytryptamine (serotonin) receptor 5B	L10073	**1.162**	0.136	**0.548**
5-hydroxytryptamine (serotonin) receptor 1A	J05276cds	**0.628**	−0.462	**0.738**

The ratio (treatment/control) was set as ≥ 1.5 ($\log_2 > 0.58$; significant up-regulation) or ≤ 0.67 ($\log_2 < -0.58$, significant down-regulation). All rations are as $\log_2$. Significant changes are in bold

GI unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats treated with vehicle, control group, *GII* 6-OHDA-lesioned rats treated chronically with L-DOPA, *GIII* 6-OHDA-lesioned rats with chronic continuous rotigotine treatment, *GIV* 6-OHDA-lesioned rats with chronic pulsatile rotigotine treatment

neurotrophin-3, insulin-like growth factor binding protein 5 (Accession no. M62781), member 2 of the tumor necrosis factor superfamily and interleukin 10 were down-regulated. Again, all these changes were normalized in rats with continuous treatment of rotigotine.

The microarray and the Q-RT-PCR analysis are summarized in Table 4. Among the eight genes tested in the SN and striatum, the patterns of gene expression determined in the microarray analysis were not confirmed in all treatment groups by Q-RT-PCR analysis. We found for all genes examined a similar pattern of gene expression in the group treated continuously with rotigotine, whereas a different expression pattern was observed under pulsatile treatment of L-DOPA (for all genes) and rotigotine (six of eight genes). Similar results were found for the neurotrophin-3 and the metabotropic 2 glutamate receptor gene in the group treated with pulsatile rotigotine. Other genes such as the insulin-like growth factor 2, the metabotropic 2 glutamate receptor, the solute carrier family 6 neurotransmitter transporter (serotonin, member 4), and the 5-hydroxytryptamine receptor 2A showed the same tendency in the Q-RT-PCR analysis as compared to the gene chip analysis but did not reach significance (Table 4). Only the changes observed regarding neurotrophin-3 reached statistical significance.

Regarding the SN, the increase of both the solute carrier family 1 glial high affinity glutamate transporter (member 2) and the 5-hydroxytryptamine receptor 1A genes showed the same tendency as observed by gene chip analysis but did not reach significance (Table 4).

Table 3 Genes in the striatum of unilateral 6-hydroxydopamine-lesioned rats that developed dyskinetic motor response following L-DOPA and pulsatile rotigotine treatment and are similarly changed

Gene name and functional group	Accession no.	Log_2(GII/GI)	Log_2(GIII/GI)	Log_2(GIV/GI)
Genes involved in growth factor metabolism				
Neurotrophin 3	E03082cds	**−2.614**	−0.174	**−0.740**
Insulin-like growth factor binding protein 5	rc_AI029920	**0.814**	−0.115	**0.864**
Insulin-like growth factor binding protein 5	M62781	**−1.178**	−0.435	**−1.238**
Insulin-like growth factor 2	X16703	**1.534**	−0.147	**3.123**
Fibroblast growth factor 5	D64085	**0.722**	−0.336	**0.637**
Genes involved in apoptosis				
Tumor necrosis factor superfamily, member 2	E02468cds	**−0.983**	−0.046	**−0.971**
Genes involved in inflammation				
Interleukin 10	AFFX-MurIL10	**−1.150**	−0.035	**−1.233**
Genes involved in neurotransmission				
Trytophan hydroxylase	X53501cds	−0.518	**−0.583**	0.229
Glutamate receptor, metabotropic 2	M92075	**0.619**	0.360	**0.525**
Solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	U89608	0.240	**0.619**	−0.201
Glutamate receptor, ionotropic, delta 2	U08256	−0.141	**−0.690**	−0.500
Glutamate receptor, ionotropic, kainate 4	X59996mRNA	-0.049	**−0.876**	0.352
Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 2A	D13211	−0.138	**−0.858**	0.214
5-Hydroxytryptamine (seretonin) receptor 7	L22558	−0.428	**−0.590**	−0.365
5-hydroxytryptamine (serotonin) receptor 5A	L10072	**1.207**	0.167	**1.294**
5-hydroxytryptamine (serotonin) receptor 2A	M64867	**1.247**	0.113	**0.702**
5-Hydroxytryptamine (seretonin) receptor 2A	L31546cds	0.065	**−0.699**	−0.348
5-Hydroxytryptamine (seretonin) receptor 1A	J05276cds	0.128	**−1.029**	0.175
Solute carrier family 6 (neurotransmitter transporter, serotonin),4	X63995	**0.991**	0.373	**1.785**

The ratio (treatment/control) was set as ≥ 1.5 ($\log_2 > 0.58$; significant up-regulation) or ≤ 0.67 ($\log_2 < -0.58$, significant down-regulation). All rations are as $\log_2$. Significant changes are in bold

GI unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats treated with vehicle, control group, *GII* 6-OHDA-lesioned rats treated chronically with L-DOPA, *GIII* 6-OHDA-lesioned rats with chronic continuous rotigotine treatment, *GIV* 6-OHDA-lesioned rats with chronic pulsatile rotigotine treatment

Discussion

The present study used microarray analysis to profile nigral and striatal gene expression changes in unilateral 6-OHDA-lesioned rats following pulsatile and continuous treatment with dopamimetic drugs. In agreement with the literature (Cenci 2002) we found that repetitive, pulsatile dopaminergic treatment (L-DOPA, rotigotine) caused a dyskinetic motor response (sensitization of rotation, AIMs). In contrast, continuous administration of rotigotine did not induce this dyskinetic response (Schmidt et al. 2008). Pulsatility is thought to be an important pharmacokinetic determinant for the induction of dyskinesia because normal striatal dopamine-receptor stimulation is thought to be continuous (Jenner 2008). Thus, the changes in gene expression occurring under pulsatile treatment could be specific indicators for adaptive changes during generation of dyskinesia.

The fact, that pulsatile administration of both L-DOPA and rotigotine caused a dyskinetic motor response whereas continuous administration of rotigotine did not, was used as selection criteria to focus on those genes related to dyskinesia and those that did not change during continuous administration. Besides receptors of the serotoninergic system (for SN see Table 2; for striatum see Table 3) and of the glutamatergic system, some genes in relation to structural adaptations were found to be changed as well. Some of the transcripts were differentially regulated in the SN and the striatum. At present, the meaning of these observations is not understood. However, the fact that some gene changes related to the pulsatile administration of rotigotine are unchanged under continuous administration could indicate that, its continuous administration could prevent plasticity changes in the nigro-striatal system. These genes, therefore, are interesting candidates for further investigations on the mechanisms inducing dyskinesia

Table 4 Comparison of gene expression data calculated by Gene Chip array and quantitative real-time RT-PCR analysis

Gene (accession no. for PCR/in chip)	Substantia nigra						Striatum					
	GII/GI		GIII/GI		GIV/GI		GII/GI		GIII/GI		GIV/GI	
	Chip ($n = 3$) Log_2	PCR ($n = 7/12$) Mean ± SD (%)	Chip ($n = 3$) Log_2	PCR ($n = 9/12$) Mean ± SD (%)	Chip ($n = 3$) Log_2	PCR ($n = 8/12$) Mean ± SD (%)	Chip ($n = 3$) Log_2	PCR ($n = 8/11$) Mean ± SD (%)	Chip ($n = 3$) Log_2	PCR ($n = 9/11$) Mean ± SD (%)	Chip ($n = 3$) Log_2	PCR ($n = 10/11$) Mean ± SD (%)
Neurotrophin 3 (NM_031073/E03082cds)	–	–	–	–	–	–	**−2.614**	7.6 ± 4.9	−0.174	77.9 ± 62.7	**−0.740**	**2.5 ± 1.7**
Insulin-like growth factor 2 (NM_031511/X16703)	–	–	–	–	–	–	***1.534***	*48.3 ± 54.2*	−0.147	426.1 ± 392.3	***3.123***	***4.11 ± 3.3***
Tumor necrosis factor superfamily; member 2 (NM_012675/E02468cds)	–	–	–	–	–	–	***−0.983***	*30.68 ± 35.4*	−0.046	173.9 ± 155.3	***−0.971***	*2.95 ± 2.5*
Glutamate receptor, metabotropic 2 (XM_343470/M92075)	–	–	–	–	–	–	***0.619***	*44.0 ± 48.7*	0.360	533.5 ± 541.6	0.525	870.3 ± 850.4
Solute carrier family 1, glial high affinity glutamate transporter, member 2 (NM_017215/rc_AI044517)	***1.742***	*5718.9 ± 5410.7*	0.510	110.2 ± 33.9	***1.801***	*108.8 ± 33.6*	–	31.7 ± 34.5	–	304.6 ± 289.8	–	2.85 ± 2.3
Solute carrier family 6, neurotransmitter transporter, serotonin, member 4 (NM_013034/X63995)	**−0.909**	–	0.168	–	**−1.703**	–	***0.991***	*29.4 ± 27*	0.373	464.7 ± 338.7	***1.785***	*93.9 ± 84.6*
5-hydroxtryptamine receptor 1A (NM_012585/J05276cds)	***0.628***	*317.2 ± 239.3*	−0.462	97.7 ± 81.4	***0.738***	*95 ± 89.1*	–	68.3 ± 85.8	–	329.9 ± 340.4	–	462.6 ± 507.2
5-hydroxtryptamine receptor 2A (NM_017254/M64867)	–	297.7 ± 195.8	–	100.1 ± 63.2	–	98.9 ± 77.4	***1.247***	*32 ± 35.8*	0.113	111.4 ± 104.2	***0.702***	*21.6 ± 20.6*

Significant values are in bold, concomitant results are underlined, dissimilar results are *cursive*. Gene chip array ratio results are presented as Log_2 of the ratio between treatment to vehicle (a value <-0.58 represents down-regulation and >0.58 represents up-regulation), quantitative RT-PCR data are represented as the respective ratios between treatment to vehicle in percent (%), 100 percent is the value for the vehicle treatment (a value <100% represents down-regulation and >100% represents up-regulation)

– , not measured in Q-RT-PCR/no significant alteration in chip; *GI* unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats treated with vehicle, control group, *GII* 6-OHDA-lesioned rats treated chronically with L-DOPA, *GIII* 6-OHDA-lesioned rats with chronic continuous rotigotine treatment, *GIV* 6-OHDA-lesioned rats with chronic pulsatile rotigotine treatment

following pulsatile but not continuous dopaminergic treatment.

Studies on post-mortem tissues from PD patients and from animals that developed dyskinetic motor responses indicate that the neurotransmitter and neuromodulator systems of the direct and the indirect pathways are altered during the expression of dyskinesia (Jenner 2008) including especially the D1 receptor (Guigoni et al. 2007; Konradi et al. 2004). However, we did not find an up-regulation of the D1 receptor gene in pulsatile-treated rats in the striatum (Table 2) but a down-regulation of the gene for the dopamine 1A receptor in the SN in pulsatile-treated rats whereas an up-regulation was observed under continuous rotigotine treatment (Table 2). In contrast to the supposed role of dopamine or glutamate receptors, our observations rather point to a more pronounced role of the serotoninergic systems for dyskinetic motor response which is in contrast to the findings of Konradi et al. (2004). The changes in the expression of mRNA related to the serotonergic system, however, are in line with a serotonergic hyperinnervation of the lesioned side (Maeda et al. 2005).

We performed Q-RT-PCR only on a representative subset of genes that were found altered in the microarray analysis. Among the eight genes tested, the expressions patterns found in the microarray analysis showed the same trend in the Q-RT-PCR analysis; however, they did not reach statistical significance with the exception of the NT3 gene. The large variability observed in the individual samples might be the cause. However, all genes in the group treated continuously with rotigotine and investigated by the Q-RT-PCR were confirmed to remain unchanged. The differences in gene expression as observed by the two methods may have resulted from multiple causes, especially technical differences: in the microarrays, pooled RNA from different animals was used for each array, while in the Q-RT-PCR analysis we analyzed the profiles for each rat separately. The different sequences of the genes used in the different analyses may also have caused an enhanced variation.

Alteration of the neurotrophin-3 mRNA was confirmed in both assays. The neurotrophin-3 mRNA expression within the striatum was decreased under pulsatile treatment with either drug, whereas under continuous rotigotine treatment the expression pattern remained unchanged. Neurotrophins are well known to be involved in growth and survival of neurons in the brain (Apfel 1999). In animals, haploinsufficiencies of the neurotrophin receptors trkB and/or trkC were reported to promote a PD-like phenotype (von Bohlen und Halbach et al. 2005). Recently, apoptosis induced-6-OHDA in PC12 cells was shown to be inhibited via pretreatment with neurotrophin-3 (Li et al. 2008). Li et al. (2008) could show that neurotrophin-3 prevented 6-OHDA-induced apoptosis in PC12 cells via activation of PI3-kinase/Akt pathway. Concomitantly, Gu et al. (2009) could demonstrate that transplantation of rat neural stem cells that express neurotrophin-3 endogenously into 6-OHDA-treated rats has higher and better behavioral improvements, regeneration of tyrosine hydroxylase positive cells number and migration distance compared to normal stem cells. This indicates a potentially important role of neurotrophin-3 also in development of dyskinesia.

In conclusion, our observations indicate that continuous and pulsatile administration of dopaminergic compounds may lead to a differential adaptation in gene expression pattern and thus point to the role of several genes in the generation of dyskinetic motor response. As these are the first observations for a differential regulation of gene expression with respect to different treatment regimens, further studies are needed to confirm the results. Nevertheless, the data support the idea that a continuous administration of dopaminergic drugs seems to be advantageous as the adaptive changes as well as the motor complications may be avoided.

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